PATENT COOPERATION TRATY

From the Japanese Patent Office
Internaitonal Preliminary Examination Authority

P C T

WRITTEN OPINION
(PCT Rule 66)

To: Minoru NAKAMURA Address: Room 646, Shin-Tokyo Bldg., No. 3-1 Marunouchi 3-Chome, Chiyoda-ku Tokyo 100 JAPAN	Date of Mailing: November 26, 2002
Applicant's or agent's file ref. Y1J0182	REPLY DUE within 2 months from the above date of mailing

International Appln. No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/JP02/02978	27/03/2002	30/03/2001

International Patent Classification(IPC)or both national classification and IPC
Int.Cl[7]C12N15/09,1/21,C12P21/02,C07K19/00,7/06,7/08

Applicant AJINOMOTO CO., INC.

1. This written opinion is the first (first, ect.)drawn by this International Preliminary Examination Authority.

2. This opinion contains indications relating to the following items:
   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.
   When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension.
   How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3.
   For the form and the language of the amendments, see Rules 66.8 and 66.9.
   Also For an additional opportunity to submit amndments, see Rule 66.4
   For the examiner's obligation to consider amendments and/or arguments see Rule 66.4 bis.
   For an informal communication with the examiner, see Rule 66.6
   If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is July 30, 2003

Name and mailing address JAPANESE PATENT OFFICE(IPEA/JP) 4-3, Kasumigaseki 3-Chome, Chiyoda-ku, Tokyo, 100, JAPAN	Authorized officer 4N9451 Emiko SAKAZAKI Telephone No. 03-3581-1101 Ex. 3488

WRITTEN OPINION | International Application No. PCT/JP 02//02978

I . Basis of the Opinion

1. This opinion has been drawn on the basis of;

[X] the international application as originally filed

[ ] the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

[ ] the claims:
pages _____, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

[ ] the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

[ ] the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the Written Opinion was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages _____

[ ] the claims, Nos. _____

[ ] the drawings, sheets/fig _____

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

WRITTEN OPINION | International Application No. PCT/JP02/02978

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or indusrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty(N)	Claims	YES
	Claims 1-11	NO
Inventive Step (IS)	Claims	YES
	Claims 1-11	NO
Industrial Applicability(IA)	Claims 1-11	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

PLEASE SEE ATTACHED PAPERS.

2. CITATION AND EXPLANATIONS

Cited Documents

Document 1: WO 93/3158 A1 (ORSAN) 1993.02.18,
& EP 551506 A1 & JP 6-502548 A & US 6027920 A

Document 2: JP 11-169182 (Mitsubishi Chemical, Co.) 1999.06.29
(No family)

Document 1 describes a system of secreting a particular protein by Corynebacterium, wherein a sequence encoding the protein is located in a region of a chromosome or a plasmid DNA, the sequence in the region is trancribed from 5' to 3' together with at least a part of a sequence encoding the signal sequence of PS1 or PS2, and the protein is secreted by introducing the part into Corynebacterium. Specifically, it is described that the protein is secreted by obtaining csp2 gene encoding PS2 of Corynebacterium glutamicum, obtaining an expression/secreting vector for PS1 functioning Corynebacterium glutamicum and expressing celA gene of Clostridium thermocellum in Corynebacterium glutamicum. It is also described that the elements assuring the secretion without changing or losing the secretion property include equivalent sequences to the signal sequence of either PS1 or PS2 signal sequence.

Document 2 describes that the efficiency of secretion was improved by replacing the amino acids of SecE which is the factor involved the secretion in coryneform

bacteria. Specifically, a fragment of SecE gene, where random mutations had been introduced into the gene encoding SecE, was into Brevibacterium flavum MJ-233 and the bacteria were cultured and were selected for those exhibiting a large halo. It is also described that by determining their nucleotide sequence, it was revealed that the amino acid at position 44 was different between the obtained SecE gene and the wild type SecE gene.

It was known before the priority date of the present application that a heterologous protein may be secreted by introducing a signal sequence of Corynebacterium and a gene encoding the heterologous protein into Corynebacterium, said signal sequence may be PS1 or PS2, and the signal sequence includes equivalent sequence such that the secretion property should not be changed or lost, as is described in document 1. Additionally, it was also known before the priority date of the present application that in the secretion of a protein using a coryneform bacterium, a protein will be efficiently secreted compared to the wild type by replacing an amino acid of a translocation machinery as a part of a plasmid used for introduction into bacteria, as is described in document 2. Therefore, since it would be obvious for those skilled in the art to improve the efficiency of secretion in the secretion of a heterologous protein by partly changing a sequence involved in the expression and secretion, which is introduced into a plasmid, those skilled in the art would easily produce a bacterial strain which is improved in the secretion efficiency compared to the wild type by introducing a mutation into the signal sequence described in document 1 and introduce it to a coryneform bacterium. Furthermore, it would be within the capability of those skilled in the art to select Corynebacterium glutamicum as a coryneform bacterium or the optimum condition for the mutant strains.

VI . Certain documents cited.

1. Certain publishd documents

Application No. Patent No.	Publication Date (day/month/year)	Filing Date (day/month/year)	Pirority date (valid claim) (day/month/year)
WO01/23591 A1 (AJINOMOTO CO., INC.)	05/04/2001	29/09/2000	30/09/1999

2. Non-written disclosures

Kind of non-written disclosure	Date of non-written disclosure (day/month/year)	Date of written disclosure referring to non-written disclosure (day/month/year)